Symposium: Molecular Self-Assembly - from in Vitro to Cellular Systems

2248-Symp
Symmetry-Based Design and Structure of Self-Assembling Protein Cages and Nanomaterials
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Nature is replete with self-assembling molecular structures having diverse cellular functions. The largest and most sophisticated types are built from many copies of one protein molecule (or a few distinct protein molecules) arranged following principles of symmetry. Well-studied viral capsids and lesser-known bacterial microcompartments provide examples of natural closed shell architectures. A long-standing engineering goal has been to design novel protein molecules to self-assemble into geometrically specific structures similar to the extraordinary structures evolved in Nature. Practical routes to this goal have been developed by using ideas in symmetry to articulate the minimum design requirements for achieving various types of symmetric architectures, including cages, extended two-dimensional layers, and three-dimensional crystalline materials. The key requirement is generally that two distinct self-associating interfaces have to be built into the designed protein molecule, following specific geometric specifications. Recent experiments have demonstrated success using two alternate strategies, one based on fusing together two simple oligomers (e.g. a dimer and a trimer) in a geometrically specific orientation, and one based on designing one new interface into a natural oligomer (which already bears one interface). The success of these strategies has been proven by determining crystal structures of several giant, self-assembling protein cages (100-200 Å in diameter), created by design. The ability to create sophisticated supramolecular structures from designed protein subunits opens the way to broad applications in synthetic biology. Design principles and strategies will be discussed, along with current successes.

2249-Symp
Sequestered: Molecular Physiology of Bacterial Microcompartments
David Savage.
University of California, Berkeley, Berkeley, CA, USA.
Complex spatial organization is a hallmark of the eukaryotic cell. More recently, it has been shown that prokaryotes possess a similar, yet unique, degree of organization. Striking examples of this include complexes such as bacterial microcompartments and encapsulins, which use protein shells to compartmentalize metabolic function in a manner analogous to classic lipid-based organelles. Although characterized structurally, there is a lack of understanding how micrompartments and encapsulins assemble, carry out their function, and are degraded in the context of a living cell. Here, I will discuss our recent work investigating these themes using the cyanobacterial carboxysome and thermophilic encapsulin as model systems.

2250-Symp
Leveraging Cell-To-Cell Variability to Understand Signal Transduction Networks
Suzanne Gaudet, Robin E.C. Lee.
Cancer Biology/Genetics, Dana-Farber Cancer Institute/Harvard Medical School, Boston, MA, USA.
Secreted ligands such as tumor necrosis factor (TNF) regulate cell behavior by triggering series of intracellular signaling events. One striking aspect of the response to many ligands is its quantitative, or sometimes qualitative, cell-to-cell variability. We are leveraging the cell-to-cell variability in the response of cancer cells to TNF to better understand the system properties of the regulation of transcription by NF-κB.
In response to TNF, intracellular signals promote relocalisation of NF-κB transcription factors from the cytoplasm to the nucleus where they promote transcription of inflammatory and stress-responsive genes. Because dysregulation of NF-κB is associated with chronic inflammatory diseases, autoimmunity and cancer, one might expect the nuclear abundance of NF-κB to be tightly regulated. Instead, the amount of nuclear NF-κB varies considerably from cell to cell, even in the absence of stimulus. To resolve this paradox and determine how transcription-inducing signals are encoded, we quantified single-cell NF-κB translocation dynamics and transcriptional responses in the same cells. We found that TNF-induced transcription correlates best with fold-change in nuclear NF-κB, not absolute nuclear NF-κB abundance. This fold-change detection property suggests that the system encodes memory of its pre-ligand state. To complement our experimental approaches we use computational modeling and have found that an incoherent feed-forward loop, from competition for binding to κB motifs, can provide the required memory. A model with competition recapitulates the distinct patterns of transcription we observed experimentally for different NF-κB-dependent genes. Fold-change detection buffers against stochastic variation in signaling molecules and explains how cells tolerate variability in NF-κB abundance and localization. Overall, our approaches provide a framework for understanding how transcriptional networks interpret and act on dynamical signals in ligand-induced cellular responses.

2251-Symp
Towards Artificial Cells in 2D
Roy Bar-Ziv.
Materials and Interfaces, Weizmann Institute of Science, Rehovot, Israel.
We shall discuss efforts to integrate artificial biological systems into solid materials. Inspired by the spatial patterns in morphogenesis and by microelectronics, we developed a biochip on which the protein synthesis and assembly is carried out in spatially segregated micro-compartments. New insights on the collective interactions occurring in dense biological matter and on the role of geometry and reduced dimensionality in controlling gene networks will be discussed.

Symposium: Applications of Quantum Mechanics to Biophysical Problems

2252-Symp
QM/MM Methods: Recent Developments and Application to Membrane Proteins and Molecular Motors
Qiang Cui, Ph.D.
Chemistry, Univ. of Wisc., Madison, Madison, WI, USA.
I'll briefly discuss recent developments of QM/MM methods in our lab, with an emphasis on methods that allow an efficient sampling of at least local motions coupled to the biochemical process of interest. Next, I'll discuss the application of these methods to address specific mechanistic questions in proton pumps and biomolecular motors. The applications highlight that calibrated QM/MM methods are valuable because they provide not only energetic/kinetic information for the relevant biochemical driving forces (e.g., ATP hydrolysis) in these biomolecular machines but also spectroscopic observables that can be compared directly to experiments. Another feature that emerges from these applications is that changing hydration level of internal protein cavities may play an important role in modulating the proton affinity of key groups and thus the timing of key chemical events. In short, the discussions highlight the advantages of an efficient QM/MM framework based on an approximate DFT method (DFTB3) and the diverse roles of water molecules in biomolecular functions.

2253-Symp
Hydrogen Tunneling, Electrostatics, and Conformational Motions in Enzyme Catalysis
Sharon Hammes-Schiffer.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
The roles of hydrogen tunneling, electrostatics, and conformational motions in enzyme catalysis will be discussed. We have developed hybrid quantum/classical molecular dynamics methods that include the quantum mechanical effects of the active electrons and transferring proton(s), as well as the motions of the entire solvated enzyme. These methods have been used to study the proton and hydride transfer reactions catalyzed by the enzymes dihydrofolate reductase (DHFR) and ketosteroid isomerase (KSI). The free energy profiles are generated along a collective reaction coordinate, and the changes in hydrogen bonding and electrostatic interactions are analyzed along the entire reaction pathway. An analysis of the simulations resulted in the identification and characterization of a network of coupled motions that extends throughout the enzyme and represents equilibrium conformational changes that facilitate the chemical reaction. Mutations distal to the active site are shown to significantly impact the catalytic rate constant by altering the conformational sampling of the entire enzyme, thereby changing the probability of sampling configurations conducive to the catalyzed reaction. We have also developed quantum mechanical/molecular mechanical methodology to calculate the vibrational frequency shifts of thiocyanate probes incorporated into the active site of an enzyme. This methodology is shown to reproduce the experimentally measured vibrational shifts upon binding of an intermediate analog to KSI for two different nitrile probe positions. Analysis of the simulations provides atomistic insight into the roles that key residues play in determining the electrostatic environment and hydrogen-bonding interactions experienced by the nitrile probe. This approach is also being used to study the vibrational shifts of nitrile probes